

What Is Claimed Is:

add a'7

1. A DNA molecule comprising a polynucleotide which encodes an RNA molecule, said RNA molecule comprising:

- 5
- (a) at least one *cis*-acting sequence element,
 - (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and
 - (c) at least one second nucleotide sequence selected from the group consisting of:

10

- (i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

15

- (iii) a sequence encoding an untranslated RNA molecule, or complement thereof.

2. A DNA molecule of claim 1, wherein the RNA-dependent RNA polymerase is of viral origin.

20

3. A DNA molecule of claim 1, wherein the RNA-dependent RNA polymerase is of alphaviral origin.

4. A DNA molecule of claim 1 which encodes an RNA-dependent RNA polymerase that has replicase activity at temperatures below 34°C and low or undetectable replicase activity at 34°C and above.

25

5. A DNA molecule of claim 1, wherein the second open reading frame of 1(c)(i) encodes a cytokine, lymphokine, tumor necrosis factor, interferon, toxic protein, prodrug converting enzyme, or other protein.

6. A DNA molecule of claim 1, wherein the second open reading frame of 1(c)(i) encodes human erythropoietin or human β -interferon.

5 7. A DNA molecule of claim 1, wherein the second nucleotide sequence of 1(c)(ii) or 1(c)(iii) encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA molecule, tRNA molecule, rRNA molecule, ribozyme.

8. A method of making a recombinant host cell comprising introducing a DNA molecule of claim 1 into a host cell.

10 9. An *in vitro* cell culture comprising a recombinant host cell produced by the method of claim 8.

10. An *in vitro* cell culture comprising a recombinant host cell comprising a DNA molecule of claim 1.

11. The cell culture of claim 10, wherein some or all of the DNA sequences of a DNA molecule of claim 1 are stably maintained in said host cell.

15 12. An RNA molecule transcribed from a DNA molecule of claim 1.

13. An alphaviral particle containing an RNA molecule of claim 12.

14. An *in vitro* cell culture comprising a recombinant host cell comprising an RNA molecule of claim 12.

20 15. An isolated nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO:1.

16. A method for producing a protein or untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one DNA molecule of claim 1 into said host cells; and
- (c) recovering said protein or untranslated RNA molecule.

17. A method for producing a protein or untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 12 into said host cells; and
- (c) recovering said protein or untranslated RNA molecule.

18. The method of claim 17, wherein the protein is erythropoietin.

19. The method of claim 17, wherein said RNA is packaged into an alphaviral particle.

20. A method for producing alphaviral particles containing an RNA molecule of claim 12 comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing into said host cells at least one DNA molecule of claim 1 having one or more open reading frames which encode alphaviral structural proteins; and
- (c) recovering said alphaviral particles.

21. A method for producing a protein in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) infecting said host cells with alphaviral particles produced

by the method of claim 20; and

- (c) recovering said protein.

22. The method of claim 21, wherein said protein is erythropoietin.

23. A method for regulating the expression of a protein or
untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one DNA molecule of claim 1 into said host cells; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature.

24. A method for regulating the expression of a protein or
untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 12 into said host cells; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature.

25. A method for regulating the expression of a protein or
untranslated RNA molecule in an individual comprising:

- (a) administering at least one DNA molecule of claim 1 to said individual; and
- (b) changing the temperature of at least a portion of said

individual from:

- (i) a permissive temperature to a restrictive temperature, or
- (ii) a restrictive temperature to a permissive temperature.

26. A method for regulating the expression of a protein or untranslated RNA molecule in an individual comprising:

- (a) administering at least one RNA molecule of claim 12 to said individual; and
- (b) changing the temperature of at least a portion of said individual from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature.

27. The method of claim 26 wherein said individual is a human.

28. A method for regulating the expression of a protein or untranslated RNA molecule in an individual comprising:

- (a) administering a recombinant host cell comprising at least one DNA molecule of claim 1 to said individual; and
- (b) changing the temperature of at least a portion of said individual from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature.

29. The method of claim 28, wherein said recombinant host cells are obtained from the same individual into which said host cells are administered.

30. The method of claim 29, wherein said recombinant host cells are keratinocytes, epithelial cells, or fibroblasts.

5 31. The method of claim 28, wherein said individual is a human.

32. A pharmaceutical composition comprising at least one DNA molecule of claim 1 and a pharmaceutically acceptable carrier.

33. A pharmaceutical composition comprising at least one RNA molecule of claim 12 and a pharmaceutically acceptable carrier.

10 34. A pharmaceutical composition comprising at least one alphaviral particle of claim 13 and a pharmaceutically acceptable carrier.

35. A genetically engineered, non-human animal having host cells containing at least one DNA molecule of claim 1.

15 36. The animal of claim 35, wherein the DNA molecule is stably integrated into the host cell genome.

37. A genetically engineered, non-human animal having host cells containing at least one RNA molecule of claim 12.

20 38. A DNA vector system comprising one or more polynucleotides which encode RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,
- (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and

(c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof.

39. A DNA vector system of claim 38, wherein the RNA-dependent RNA polymerase is of viral origin.

40. A DNA vector system of claim 38, wherein the RNA-dependent RNA polymerase is of alphaviral origin.

41. A DNA vector system of claim 38 which encodes an RNA-dependent RNA polymerase that has replicase activity at temperatures below 34°C and low or undetectable replicase activity at 34°C and above.

42. A DNA vector system of claim 38, wherein the second open reading frame of 1(c)(i) encodes a cytokine, lymphokine, tumor necrosis factor, interferon, toxic protein, prodrug converting enzyme, or other protein.

43. A DNA vector system of claim 38, wherein the second open reading frame of 1(c)(i) encodes human erythropoietin or human β -interferon.

44. A DNA vector system of claim 38, wherein the second nucleotide sequence of 1(c)(ii) or 1(c)(iii) encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA molecule, tRNA molecule, rRNA molecule, ribozyme.

45. A method of making a recombinant host cell comprising introducing at least one polynucleotide of claim 38, into a host cell.

46. An *in vitro* cell culture comprising a recombinant host cell produced by the method of claim 45.

5 47. An *in vitro* cell culture comprising a recombinant host cell comprising at least one polynucleotide of claim 38.

48. The cell culture of claim 47, wherein some or all of the polynucleotide sequences of claim 38 are stably maintained in said host cell.

49. A composition comprising one or more RNA molecules transcribed from one or more polynucleotides of the vector system of claim 38,

10 50. An alphaviral particle containing at least one RNA molecule of claim 49.

51. An *in vitro* cell culture comprising a recombinant host cell comprising at least one RNA molecule of claim 49.

15 52. A method for producing a protein or untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one polynucleotide of claim 38 into said host cells; and
- 20 (c) recovering said protein or untranslated RNA molecule.

53. A method for producing a protein or untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one RNA molecule of claim 49, into

said host cells; and

- (c) recovering said protein or untranslated RNA molecule.

54. The method of claim 53, wherein the protein is erythropoietin.

55. The method of claim 53, wherein said RNA is packaged into an alphaviral particle.

56. A method for producing alphaviral particles containing an RNA molecule of claim 50 comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing into said host cells at least one polynucleotide of claim 38 having one or more open reading frames which encode alphaviral structural proteins; and
- (c) recovering said alphaviral particles.

57. A method for producing a protein in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) infecting said host cells with alphaviral particles produced by the method of claim 56; and
- (c) recovering said protein.

58. The method of claim 57, wherein said protein is erythropoietin.

59. A method for regulating the expression of a protein or untranslated RNA molecule in a recombinant host cell comprising:

- (a) growing host cells under suitable culture conditions;
- (b) introducing at least one polynucleotide of claim 38 into said host cells; and
- (c) changing the temperature of the host cell culture from:
 - (i) a permissive temperature to a restrictive

temperature, or

(ii) a restrictive temperature to a permissive temperature.

5 60. A method for regulating the expression of a protein or untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;
(b) introducing at least one RNA molecule of claim 49 into said host cells; and

10 (c) changing the temperature of the host cell culture from:
(i) a permissive temperature to a restrictive temperature, or
(ii) a restrictive temperature to a permissive temperature.

15 61. A method for regulating the expression of a protein or untranslated RNA molecule in an individual comprising:

(a) administering at least one polynucleotide of claim 38 to said individual; and

(b) changing the temperature of at least a portion of said individual from:

20 (i) a permissive temperature to a restrictive temperature, or
(ii) a restrictive temperature to a permissive temperature.

25 62. A method for regulating the expression of a protein or untranslated RNA molecule in an individual comprising:

(a) administering at least one RNA molecule of claim 49 to said individual; and

(b) changing the temperature of at least a portion of said individual from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature.

5 63. The method of claim 62, wherein said individual is a human.

64. A method for regulating the expression of a protein or untranslated RNA molecule in an individual comprising:

(a) administering a recombinant host cell comprising at least one polynucleotide of claim 38 to said individual; and

(b) changing the temperature of at least a portion of said individual from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature.

65. The method of claim 64, wherein said recombinant host cells are obtained from the same individual into which said host cells are administered.

66. The method of claim 65, wherein said recombinant host cells are keratinocytes, epithelial cells, or fibroblasts.

20 67. The method of claim 64, wherein said individual is a human.

68. A pharmaceutical composition comprising at least one polynucleotide of claim 38 and a pharmaceutically acceptable carrier.

69. A pharmaceutical composition comprising at least one RNA molecule of claim 49 and a pharmaceutically acceptable carrier.

70. A pharmaceutical composition comprising at least one alphaviral particle of claim 50 and a pharmaceutically acceptable carrier.

71. A genetically engineered, non-human animal having host cells containing at least one polynucleotide of claim 38,

5 72. The animal of claim 71, wherein the polynucleotide is stably integrated into the host cell genome.

73. A genetically engineered, non-human animal having host cells containing at least one RNA molecule of claim 49,

10 74. A composition comprising one or more RNA molecules, said RNA molecules comprising:

(a) at least one *cis*-acting sequence element,
(b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and

15 (c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

20 (ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof.